CHAPTER V

SEASONAL ALTERATIONS IN THE ACTIVITY LEVELS OF PHOSPHOMONOESTERSES IN THE LIVER AND GONADS OF NORMAL AND ADRENAL MANIPULATED WILD PIGEONS, COLUMBA LIVIA

Phosphatases are thought to be involved in various aspects of cellular metabolism and have therefore drawn considerable attention. As far as their specific biological functions are concerned relatively less is understood despite their ubiquitous distribution. Phosphatases are classified into phosphomonoesterases, phosphodiesterases and pyrophosphatases of which phosphomonoesterases (acid and alkaline phosphatases) are the most widely distributed ones. Since these phosphatases hydrolyze a number of phophate esters, they are termed as non-specific phosphatases. Apart from hydrolysis of phosphate esters and dephosphorylating mechanisms, are considered to be associated with several prominent aspects of cell and tissue physiology. Some of the important functions attributed to acid phospotase are its role as a hydrolytic enzyme, in the regulation of pyridoxal phospate requiring enzymes, involvement in steroid transport, in vitamin B_6 metabolism, in lipid metabolism, in cellular differentiation and disintegration of tissue components, in several metabolic reactions of testes and other reproductive organs and in absorption and phosphorylating

mechanisms(Burstone, 1962; Shettan, 1964; Andrews and Turner, 1966; Dipietro and Zengerle,1967; Pearse, 1968; Klockars and Wageliu& 1969; Heinrikson,1969; Cohen, 1970; Blank and Snyder,1970; Serrano et al.,1976). Similarly, some of the general but important functions attributed to alkaline phosphatase are its association with transmembrane transport mechanisms, process of calcification and growth, differentiation, and metabolism of DNA and carbohydrates (Moog, 1946; Sols, 1949; Bradfield, 1951; Rogers, 1960; Rosenthal et al.,1960; Simkiss, 1964, Rackallio, 1970). These enzyems are known to occur in isozymic forms which reflect and unique but as yet undefined metabolic function for each of them.

There are a few studies which have tried to evaluate the functional association of phosphatases with gonads and accessory sexual organs. (Kugler et al.,1956; Bialy and Pincus, 1967; Chinoy et al.,1973; Chinoy and Sheth, 1977; Guha and Vanha, 1983; Kornblatt et al.,1983). Tanabe and Wilcox (1961) in their study on serum alkaline phosphatase reported it to be under the control of thyroxine as well as oestrogen to some extent. Further, Patel (1976) and Patel (1982) reported alterations in the activity levels of both acid and alkaline phosphatases in several tissues of birds in relation to reproductive activities. Pituitary-adrenal axis has also been noted to influence the activity

of phosphatases during the functional differentiation of duodenum in suckling mouse (Moog, 1965). On a parallel line of theoretical thinking, it was speculated that the activity of phosphatases could play an important role in the cyclic functioning of avian gonads. This prompted the idea of studying the activity levels of phosphatases in the gonads during the annual reproductive cyclicity of wild pigeons on the one hand, and the influence of adrenal suppression during the breeding months and activation during the non-breeding months, on the other hand. The activity levels of both acid and alkaline phosphatases in the liver was also studied due to the involvement of this organ in many metabolic homeostatic activities associated with avian gonadal cyclicity.

MATERIALS AND METHODS

As outlined in Chapter I

RESULTS

Results obtained are depicted in tables 1 - 4 and figures (1 - 8). The tables and figures represent cumulative values of testis and ovary put together as no remarkable sex difference in the activity levels of the two enzymes was discernible.

Changes in Normal Birds

Both liver and gonads exhibited seasonal variations in the activity levels of the two phosphatases. A progressive

TABLE-1 : SEASONAL CHANGES IN HEPATIC ACID PHOSPHATASE ('µ' MOLES PARA NITRO PHENOL RELEASED/100 mg PROTEIN/30 MINUTES) OF NORMAL AND EXPERIMENTAL PIGEONS, C. LIVIA (± S.D.).

REPRODUCTIVE PHASES	NORMAL	DEXAMETHASONE			ACTH	CORTICOSTERONE			
		80µg	120µg	160µg	0.5 I.U.	1µgM	1µgE	3µgM	3µgE
RECRUDESCENT	150.32 ±35.8	106.26+ ±14.2	140.17 ±2.60	123.97* ±5.5	-	-	-	-	-
BREEDING	102.65 ±18.21	91.78 ±11.66	102.45 ±7.84	109.14 ±9.82	-	-	-	-	-
REGRESSION	54.24 ±10.51	-	-	-	140.40** ±36.10	67.31* ±12.8	90.96*** ±11.6	66.59 ±11.0	70.56* ±14.31

+ P<0.01 * P<0.05 ** P<0.005 *** P<0.0005

M - MORNING E - EVENING

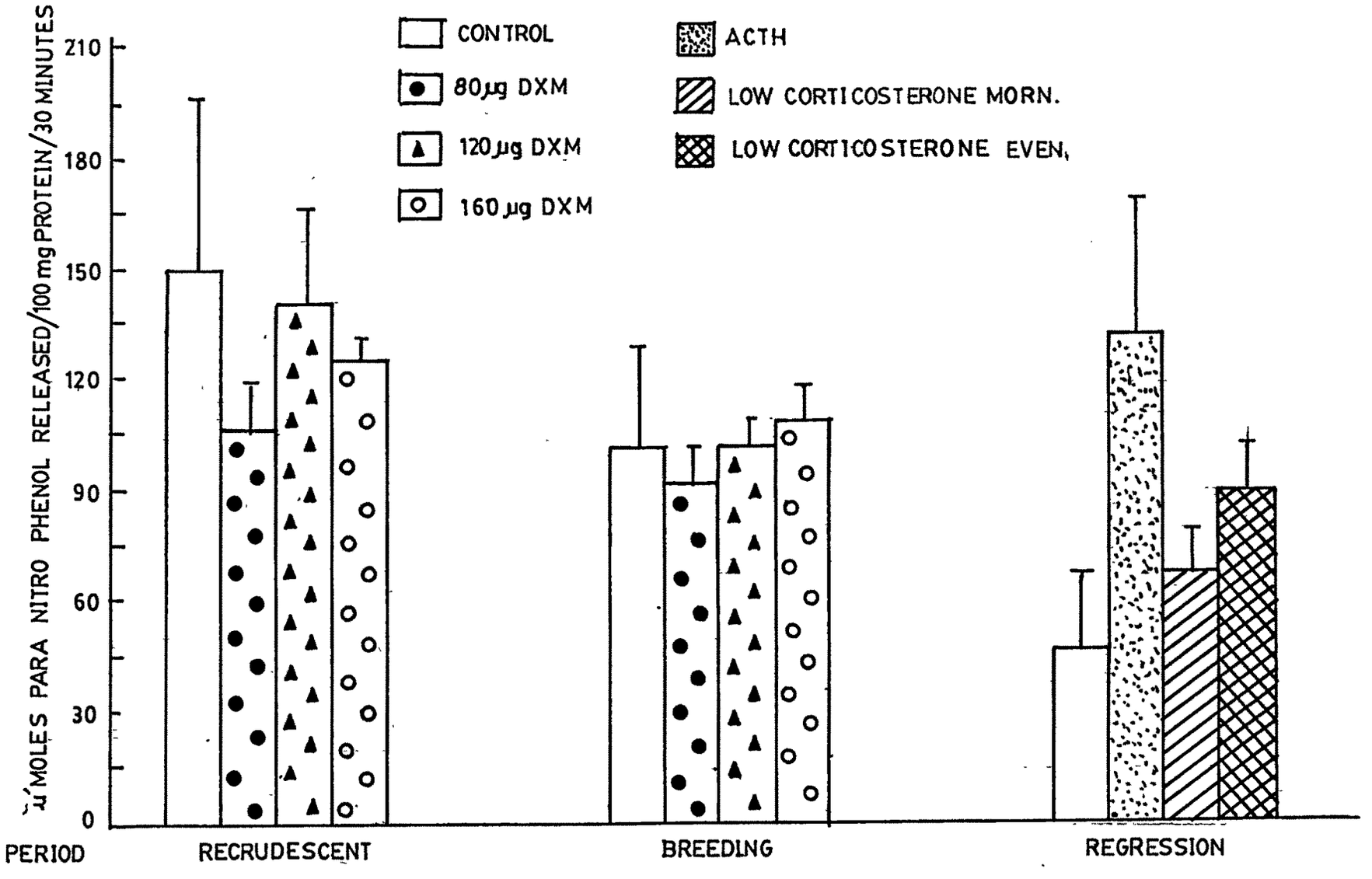

FIG. 1. CHANGES IN HEPATIC ACID PHOSPHATASE ACTIVITY

TABLE-2 : SEASONAL CHANGES IN GONADAL ACID PHOSPHATASE (μ MOLES PARA NITRO PHENOL RELEASED/100 mg PROTEIN/30 MINUTES) OF NORMAL AND EXPERIMENTAL PIGEON, C.LIVIA ($\pm$ S.D.).

REPRODUCTIVE PHASES	NORMAL	DEXAMETHASONE			ACTH	CORTICOSTERONE			
		80µg	120µg	160µg	0.5 I.U.	1µgM	1µgE	3µgM	3µgE
RECRUDESCENT	90.35 $\pm$13.34	73.46^{+} $\pm$7.32	66.86** $\pm$12.60	66.07** $\pm$19.98	-	-	-	-	-
BREEDING	76.27 $\pm$15.16	59.14* $\pm$7.97	78.66 $\pm$13.25	63.96* $\pm$11.91	-	-	-	-	-
REGRESSION	57.05 $\pm$13.6	-	-	-	65.12 $\pm$13.26	72.12* $\pm$6.84	55.66 $\pm$9.18	32.6** $\pm$5.7	35.41** $\pm$5.94

* $P < 0.05$ ** $p < 0.005$

M - MORNING E - EVENING

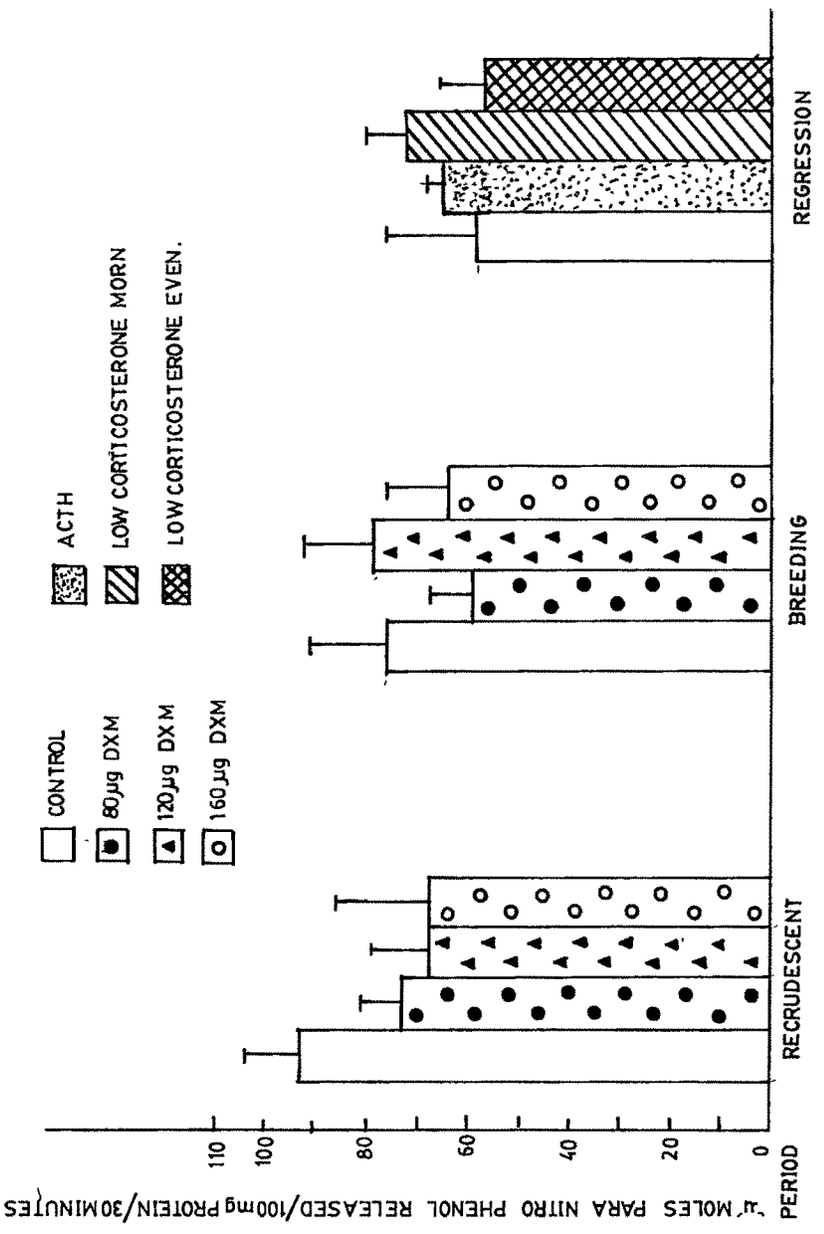

FIG. 2. CHANGES IN GONAD ACID PHOSPHATASE ACTIVITY

TABLE-3 : SEASONAL CHANGES IN HEPATIC ALKALINE PHOSPHATASE (μ MOLES PARA NITRO PHENOL RELEASED/100 mg PROTEIN/30 MINUTES) OF NORMAL AND EXPERIMENTAL PIGEONS, C.LIVIA (± S.D.).

REPRODUCTIVE PHASES	NORMAL	DEXAMETHASONE			ACTH	CORTICOSTERONE			
		80μg	120μg	160μg	0.5 I.U.	1μgM	1μgE	1μgM	3μgE
RECRUDESCENT	8.90 ±2.39	8.37 ±1.46	12.47** ±2.29	11.40* ±2.95	-	-	-	-	-
BREEDING	5.12 ±1.33	5.28 ±1.9	10.32++ ± 2.38	6.20* ±0.95	-	-	-	-	-
REGRESSION	6.17 ±1.28	-	-	-	4.77* ±1.12	6.62 ±2.14	7.86 ±1.6	4.89* ±1.29	5.20* ±1.28

++ $P < 0.001$ * $P < 0.05$ ** $P < 0.005$

M - MORNING E - EVENING

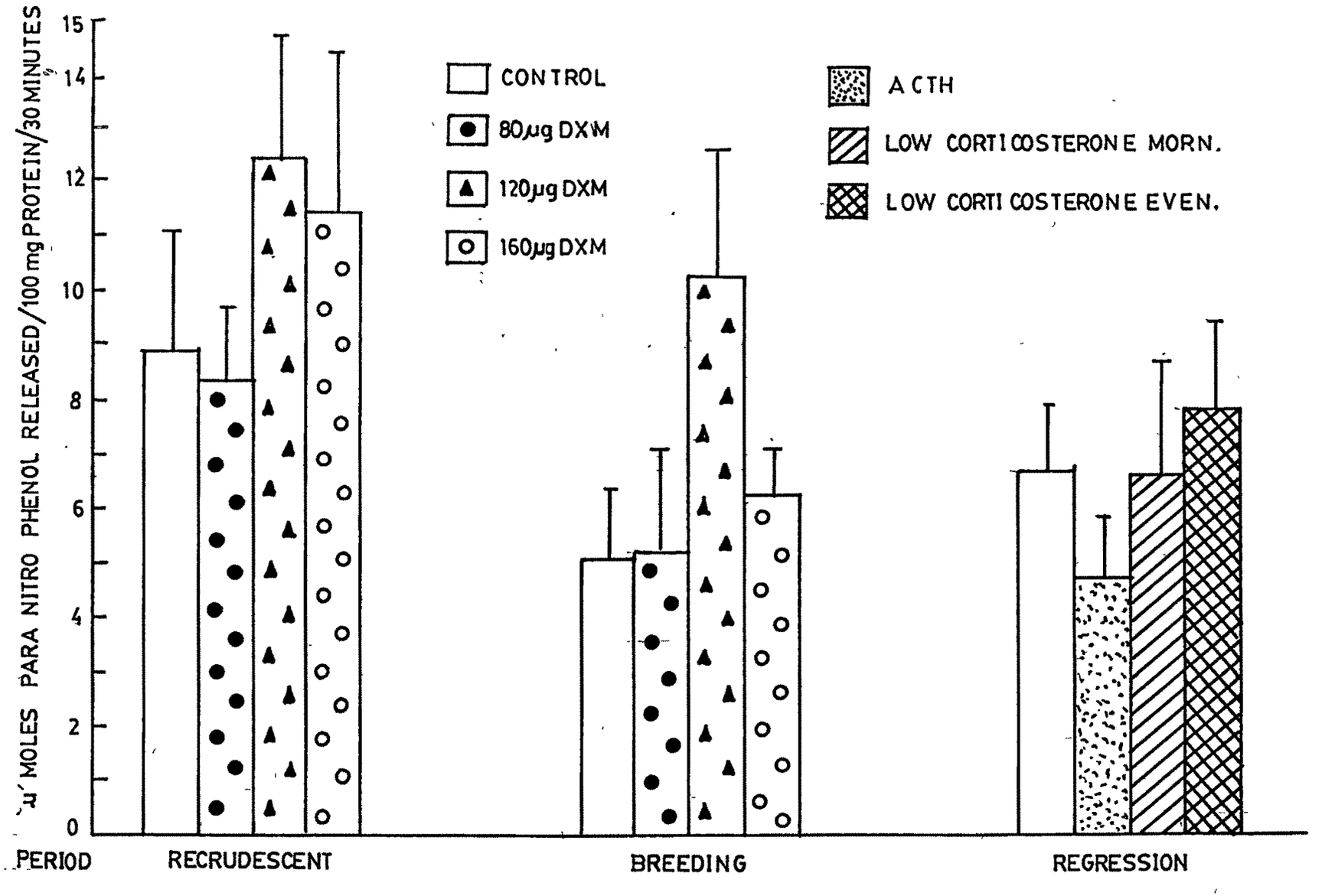

FIG. 3. CHANGES IN HEPATIC ALKALINE PHOSPHATASE ACTIVITY

TABLE-4 : SEASONAL CHANGES IN GONADAL ALKALINE PHOSPHATASE (μ MOLES PARA NITRO PHENOL RELEASED/100 mg PROTEIN/30 MINUTES) OF NORMAL AND EXPERIMENTAL PIGEONS, C.LIVIA (± S.D.).

REPRODUCTIVE PHASES	NORMAL	DEXAMETHASONE			ACTH	CORTICOSTERONE			
		80μg	120μg	160μg	0.5 I.U.	1μgM	1μgE	3μgM	3μgE
RECRUDESCENT	41.32 ±9.93	25.97^{+} ±7.11	34.14* ±8.4	28.63* ±4.71	-	-	-	-	-
BREEDING	34.87 ±3.22	6.19*** ±0.91	9.40*** ±3.5	9.05*** ±2.9	-	-	-	-	-
REGRESSION	20.61 ±2.08	-	-	-	22.68 ±4.9	37.31^{++} ±9.33	18.31 ±3.0	15.02** ±3.38	12.78*** ±3.14

+ $p < 0.01$ * $P < 0.05$ ** $P < 0.005$ *** $P < 0.0005$

M - MORNING E - EVENING

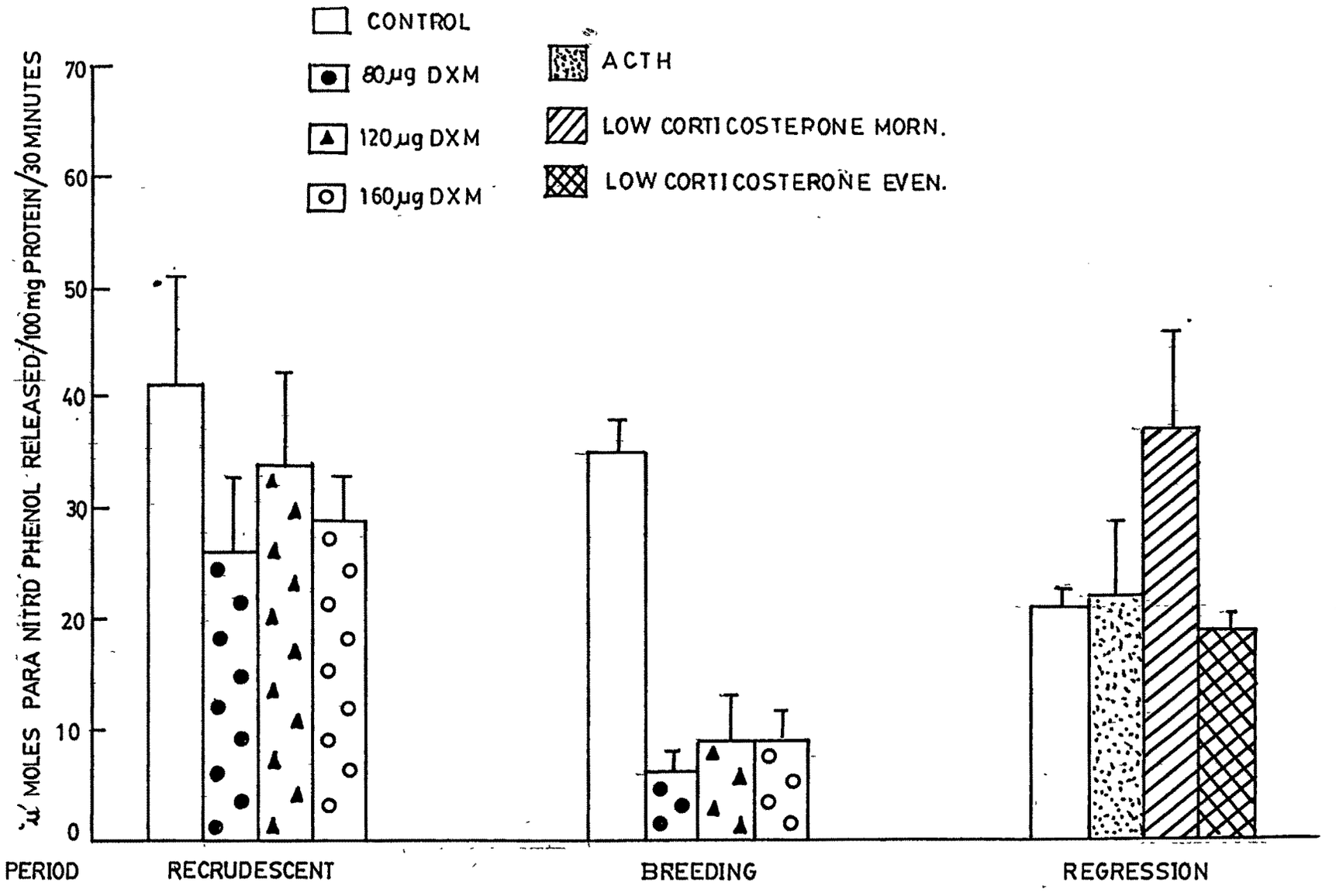

FIG.4: CHANGES IN GONADAL ALKALINE PHOSPHATASE ACTIVITY

suppression during the recrudescent and breeding phases led to reduced acid phosphatase activity in the liver and gonads. Adrenal activation during the regression phase brought about a reverse set of changes which resulted in increased level of activity of acid phosphatase. Response of alkaline phosphatase activity to the experimental manipulation differed in the two tissues under study. Whereas adrenal suppressed birds revealed an increased alkaline phosphatase activity in liver, gonads revealed decreased enzyme activity. Adrenal activation (ACTH, LCM and LCE) reversed the situation with liver depitcting decreased enzyme activity and the gonads increased enzyme activity. However, HCM and HCE brought about changes similar to those found in adrenal suppressed birds during recrudescence and breeding. This holds true for gonads only as the alterations in liver did not depict such a trend.

DISCUSSION

Season specific alterations in gonadal activity are known to be mediated and brought about by changes in gonadotrophic secretions from the pituitary. Gonadal activity is also known to be affected by the interactions of other endocrine principles, whereby the activity of other glands can modulate the secretion of gonadotropic as well as gonodal steroids or vice versa. Such complex interactions

of endocrine secretions however ensure successful breeding by bringing about the requisite alterations in the metabolic profile. In the present study, the adrenal-gonad interrelationship is being assessed by experimental manipulations of adrenal activity and the resultant effect on the activity levels of phosphatases in the gonads and liver. Functional association of phosphatases with the active state of the gonads can be inferred from the present observations of high enzyme activity in the gonads and liver during recrudescence and breeding and reduced activity during the regression phase. Available reports on mammals and birds indicate higher incidence of acid phosphatase in comparison to alkaline phosphatase in both liver and gonads (Thorbeck et al., 1960; Patel, 1982; Patel and Ramachandran, 1986). High acid phosphatase activity recorded in liver herein during the recrudescent and breeding phases could be associated with the gearing up of metabolic functions associated with reproductive activity in the light of the reported role of acid phosphatase in several metabolic functions such as protein synthesis, steroid transport and lipid metabolism (Dipietro and Zengerle, 1967; Blank and Snyder, 1970), similarly, high activity of acid phosphatase in the gonads during recrudescence could be correlated with the mopping up of cell debris accumulated in the regressed gonads. One of the earlier functions attributed to acid

phosphatase because of its association with lysosomes was hydrolytic one (Duve et al., 1962), and its involvement in cellular differentiation and disintegration has also been suggested (Burstone, 1962; Heinrikson, 1969). More or less a steady high level of acid phosphatase activity recorded in the present study during the breeding phase as well could probably aid in processes like sperm maturation, differentiation and nutrition (Burstone, 1962; Serrano et al., 1976). Functional significance of acid phosphatase of a similar nature has been attributed by Dasgupta and Bhattacharyya (1984) in their study on red vented bulbul, who also incidentally observed high acid phosphatase activity during the active phase of gonads. Reduced levels of the enzyme in liver and gonads during the regression phase suggests a loss or reduction of the seasonal functional attributes associated with gonadal cyclicity. Importance of acid phosphatase in active spermatogenesis can be realised from the observation of Guha and Vanha-perttula (1983) of steadily increasing acid phosphatase isoenzyme forms III & IV from 3 weeks of age in the mouse testis concomitant to histologically noticeable appearance of spermatids and sperms. In this context, the herein observed high acid phosphatase activity during the recrudescent phase apparently suggests the functional involvement of acid phosphatase in the commencement and/or establishment of spermatogenesis. The decreased enzyme

activity in the liver and gonads of adrenal suppressed birds along with the associated shrinkage of gonad (Chapters II & III) reflect the inability of these birds to bring about the requisite adaptive modulations for maintaining the functional ability of gonads. The increased hepatic and gonadal acid phosphatase activity and gonadal enlargement noted to occur in ACTH/corticosterone treated birds during the non-breeding phase are suggestive of the involvement of adrenal corticosteroids in regulating reproductive ability of tropical wild pigeons.

Alkaline phosphatase, a membrane bound enzyme has been reported to be involved in transmembrane transport of metabolites, ions and phosphate molecules in phospholipid metabolism and in nucleic acid synthesis (Chevremont and Firket, 1953; Danielli, 1954; Allen and Slater, 1956). High levels of alkaline phosphatase activity in both liver and gonads during the recrudescent and breeding phases as compared to the non-breeding phase reflect the importance of transport of substances in and out of the tissues prior to and during the breeding season. Reduction in alkaline phosphatase activity in the regression phase is in this vein indicative of decreased functional demands. High alkaline phosphatase activity in the ovarian follicles has been reported by Gasparska et al. (1982) in the laying

hen. Presence of alkaline phosphatase and its characterization in rat testes has been reported by Kornblatt et al. (1983). Adrenal suppression in the present study led to increased alkaline phosphatase activity in the liver while it was decreased in the gonads. High activity of this enzyme in both liver and gonads has been the feature in normal birds during recredescence and breeding. The decreased activity in the gonads of dxm treated birds along with the regressed state (Chapters II & III) resemble the conditions of an otherwise normal non-breeding phase. Obviously, an alteration in alkaline phosphatase activity seems to have a definite relation with the histomorphological status of the gonads. This once again is emphasised by the observed gonadal enlargement (Chapters II & III) and increase in alkaline phosphatase activity occurring in birds treated with ACTH/corticosterone during the regression phase. However, neither HCM and HCE nor LCE treatments in general could bring about any significant changes with regard to phosphomonoesterase activity, thereby suggesting dose and time specificity of adrenal corticosteroids in bringing about normal seasonal enzymic modulations.

From the present observations it can be inferred that while acid phosphatase activity is associated with germ cell differentiation and sperm metabolism, alkaline

phosphatase activity is concerned with selective transport of substances in relation to gametogenesis and gamete maturation. Further, the involvement of adrenal corticosteroids in inducing the adaptive modulations of phosphatase activity cannot be over-ruled in the light of the observed alterations in the experimental birds. This observed effects could either denote a direct action of adrenal steroid on gonads or an indirect one by influencing the hypothalamo-hypophysial axis.

S U M M A R Y

Nonspecific phosphomonoesterases were quantitatively assayed in the gonads and liver of normal and adrenal manipulated pigeons on a seasonal basis. Both the enzymes depicted high levels of activity during the recrudescent and breeding months and low levels during the non-breeding months. Whereas the high levels of activity of these enzymes are correlatable with increased lysosomal activity and material transport related to gonadal activation and gametogenesis, their low activity in the non-breeding phase attests to the loss of these functions. Birds whose adrenals were suppressed by dexamethasone during the reproductively active phases showed reduced enzyme activity coupled with induced gonadal regression and, birds whose adrenal activity was elevated by exogenous administration of ACTH/corticosterone in the non-breeding phase recorded increased enzyme activity coupled with gonadal activation. Apparently, the involvement of corticosteroids in modulating the activity of phosphatases either in a cause or effect manner can be presumed.